Pathology – Research and Practice 211 (2015) 421–425

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Comparison of the 2007 and 2013 ASCO/CAP evaluation systems for HER2 amplification in breast cancer

Xiaohong Pu, Jiong Shi, Zhiwen Li, Anning Feng, Qing Ye*

Department of Pathology, Nanjing University Medical School Affiliated Drum Tower Hospital, 321 Zhongshan Road, Nanjing, Jiangsu 210008, China

ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 16 August 2014
Accepted 22 September 2014

Keywords:
Breast cancer
HER2
Chromosome 17 polysomy
2013 ASCO/CAP scoring criteria

ABSTRACT

It has been proven that chromosome 17 centromere (CEP17) amplification causes misleading human epidermal growth factor receptor 2 (*HER2*) gene fluorescence in situ hybridization (FISH) results, precluding anti-HER2-based therapy in some patients with breast carcinoma. We used the 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) scoring criteria to evaluate HER2 amplification status in 175 cases of breast carcinoma with chromosome 17 polysomy. We used immunohistochemistry (IHC) to determine the HER2 amplification status, and 2-color FISH to detect CEP17, and reviewed the results of initial evaluation using the 2007 ASCO/CAP criteria. Of the 175 cases, 17, 95, and 63 were IHC 0/1+, 2+, and 3+, respectively. Evaluation of IHC HER2 status according to the 2013 ASCO/CAP criteria identified significantly more HER2-positive cases compared to cases evaluated using the 2007 criteria ($p < 0.05$). When the FISH results were evaluated in parallel with the 2013 criteria, we found that 22 cases were not HER2-negative despite the presence of polysomy 17, which, according to the 2013 criteria, indicates HER2-positive status. Our findings indicate that in breast carcinoma, HER2 status in the presence of polysomy 17 may vary with the scoring criteria used. In turn, performing FISH and evaluating samples using the 2013 ASCO/CAP criteria means that more patients with breast cancer may be appropriate for targeted treatment with trastuzumab, potentially improving their outcome.

Introduction

Breast cancer is the most common cancer in women and the second most common cancer worldwide [1]. In the last decade, targeted therapy in breast cancer has become part of routine clinical protocols all over the world. Trastuzumab, a humanized monoclonal antibody that targets human epidermal growth factor receptor 2 (HER2), is routinely used to treat patients with breast carcinoma who overexpress HER2 [2,3]; when combined with chemotherapy in the metastatic setting, trastuzumab improves progression-free survival and overall survival by years [4]. Other HER2-targeting drugs (e.g., the kinase inhibitor lapatinib [5], the antibody pertuzumab [6], the antibody–drug conjugate ado-trastuzumab emtansine [T-DM1] [7]) have been approved for use in the treatment of HER2-positive metastatic breast cancer. At the same time, it has been shown that lapatinib (when added to paclitaxel) [8] and pertuzumab (as a single agent) [9] offer no clinical benefit to patients with HER2-negative metastatic disease. HER2 encodes a 185-kDa receptor tyrosine kinase, activating signaling pathways that stimulate cell proliferation and survival, including the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) and mitogen-activated protein kinase (MAPK) pathways [10]. Approximately 20% of breast cancers overexpress HER2, caused by amplification of the erbB2 oncogene [11–14]. As a marker of aggressive disease, HER2 overexpression is an independent predictor of decreased recurrence-free survival, breast cancer-related survival, and overall survival [15,16]. The development of HER2-targeting therapy has revolutionized the treatment of HER2-positive breast cancer such that we may consider HER2 overexpression a positive predictor of improved outcome.

Studies worldwide have identified the significant benefit of first-line trastuzumab therapy in conjunction with surgery and cytotoxic chemotherapy for treating HER2-positive breast carcinoma [17,18]. Thus, accurate HER2 testing to ensure that the right patient receives the right treatment is now more critical than ever [19–21]. Currently, we evaluate HER2 status mainly with immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH); IHC analysis is usually used as the primary assay, and reflex FISH is performed for a specific subset of IHC results (e.g., 1+ or 2+); other laboratories primarily use FISH [22,23]. The 2013 ASCO/CAP (American Society of Clinical Oncology/College

* Corresponding author.
E-mail address: yeqing1998@gmail.com (Q. Ye).

http://dx.doi.org/10.1016/j.prp.2014.09.010

of American Pathologists) guideline defines HER2-positive breast carcinoma as tumors containing >10% of cells with complete and intense membrane staining by IHC. FISH-positive breast carcinoma is defined as average HER2 copy number ≥ 6.0 signals/cell or average HER2 copy number ≥ 4.0 signals/cell and HER2/chromosome 17 centromere (CEP17) ratio ≥ 2.0 [24]. In comparison, the 2007 ASCO/CAP guideline uses a cutoff value of HER2/CEP17 ratio > 2.2 to define HER2 overexpression [24–26]. The 2013 criteria benefits many more patients in terms of the targeted drugs they may potentially receive, especially patients with chromosome 17 polysomy (polysomy 17) as identified by dual-probe FISH. In terms of *HER2* gene assessment, it has been proven that CEP17 amplification causes misleading HER2 FISH results [27–31], precluding anti-HER2-based therapy for some patients. In this study, we used the 2013 ASCO/CAP scoring criteria to evaluate HER2 amplification status in breast carcinoma with polysomy 17.

Materials and methods

Patients

The study involved 175 cases with primary invasive breast cancer. Samples were obtained after the patients had provided informed consent; the Nanjing Drum Tower Hospital Ethics Committee approved the study. The HER2 IHC was determined and we reviewed the HER2 status of the archived samples, and analyzed the tumors according to the 2007 and 2013 ASCO/CAP guidelines.

IHC

Each tissue sample was fixed immediately in 10% neutral buffered formalin for 6–48 h, and then paraffin-embedded. Sections (4 μm) were deparaffinized routinely, rehydrated, and retrieved. HER2 expression was detected using 1:300 polyclonal antibody A0485 (DakoCytomation, Glostrup, Denmark) overnight at 4 °C. Positive and negative controls were run together with the test sample. Using the 2007 ASCO/CAP criteria, HER2 expression was scored as follows: 0 = no staining; 1+ = weak, incomplete membrane staining in >10% of tumor cells; 2+ = weak to moderately complete membrane staining in >10% of tumor cells; 3+ = strong, complete membrane staining in >30% of tumor cells [24–26]. In the 2013 ASCO/CAP scoring criteria, IHC 3+ = complete, intense staining of >10% of tumor cells; IHC 2+ = circumferential, incomplete and/or weak/moderate membrane staining in >10% of tumor cells or complete and circumferential intense membrane staining in ≤10% of tumor cells; IHC 1+ = faint/barely perceptible incomplete membrane staining in >10% of tumor cells; IHC 0 = no staining or incomplete and faint/barely perceptible membrane staining in ≤10% of tumor cells [24]. We used the 2007 guidelines to evaluate HER2 IHC.

FISH

Two-color FISH was performed on 2-μm thick sections from formalin-fixed, paraffin-embedded tissue sections from all 175 cases. Before hybridization, sections were deparaffinized, dehydrated in 100% ethanol, and air-dried. Commercially available, locus-specific HER2 probe (190-kb SpectrumOrange directly labeled fluorescent DNA probe) and CEP17 probe (5.4-kb Spectrum Green directly labeled fluorescent DNA) were used according to the manufacturer's recommendations (Jinpujia, Beijing, China). We scored 30 nuclei per sample, and recorded the number of HER2 (red) and CEP17 (green) signals according to the 2007 ASCO/CAP criteria. Gene amplification was indicated when the HER2/CEP17 ratio > 2.2; amplification was equivocal when 1.8 ≤ HER2/CEP17 ratio ≤ 2.2, and negative when HER2/CEP17 ratio < 1.8 [24–26]. The 2013 ASCO/CAP criteria uses HER2/CEP17 ratio ≥ 2.0 (Fig. 1a and b) or HER2/CEP17 ratio < 2.0 but average HER2 copy number ≥ 6.0 signals/cell (Fig. 1c) to indicate the mean HER2 amplification for 20 cells. According to the 2013 guidelines, HER2/CEP17 ratio < 2.0 and average HER2 copy number ≥ 4.0 and <6 signals/cell indicated equivocal amplification (Fig. 1e and f); HER2/CEP17 ratio < 2.0 and average HER2 copy number < 4 signals/cell indicated negative amplification (Fig. 1d) [24]. Polysomy 17 was defined as >1.86 CEP17 signals per nucleus [27–31].

Statistical analysis

A nonparametric chi-square test was used for testing associations between variables and p values < 0.05 were considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences software (v17.0; SPPS Inc., Chicago, IL).

Results

Clinicopathological characteristics of the cohort

All 175 patients were women, the age range was 31–78 years (mean 53 years), and all patients had invasive breast carcinoma.

Comparison of IHC and FISH for HER2 status

More than half of the cases were IHC 2+ (95 cases, 54.3%). The remaining cases included 17 IHC 0 or IHC 1+ cases (9.7%), and 63 IHC 3+ cases (36.0%). In the 17 IHC 0/1+ cases, 16 were HER2-negative by FISH based on the 2007 ASCO/CAP guidelines; FISH determined that one case was HER2-equivocal. Of the 95 patients identified as IHC 2+, 61 were classified as *HER2*-non-amplified and 34 were *HER2*-amplified according to the 2007 guideline. Of 63 IHC 3+ patients, 56 were *HER2*-amplified, and seven were HER2-negative by FISH. In the IHC 2+ cases, FISH determined that a much larger proportion was HER2-negative than HER2-positive (64.8% vs. 35.2%). We obtained different results when we reevaluated HER2 status using the 2013 ASCO/CAP scoring criteria. As shown in Table 1, there were significantly more HER2-positive cases, which were, in order of case increases: IHC 2+ (from 34 to 43 cases, $p < 0.05$), IHC 3+ (from 56 to 60, $p > 0.05$), IHC 1+ (increase from 0 to 3, $p < 0.05$). There was also a significant increase in HER2-equivocal cases, where IHC 2+ cases increased from 0 to 5, followed by IHC 1+ cases. Correspondingly, there were fewer *HER2*-non-amplified cases (Table 1).

Comparison between 2007 and 2013 ASCO/CAP guidelines for FISH-derived HER2-negative status accompanied by polysomy 17

According to the 2007 ASCO/CAP guideline, HER2-positive status by FISH was defined as HER2/CEP17 ratio > 2.2, but based on the 2013 ASCO/CAP guideline, many HER2-non-amplified cases with polysomy 17 should be redefined, given that previously defined HER2-negative cases may be defined as HER2-amplified according to the 2013 guideline. There was polysomy 17 in 100 (57.1%) of the 175 patients, of which 48 were defined as *HER2*-non-amplified based on the 2007 criteria. Using the criterion of ≥6 HER2 signals per nucleus to denote positive amplification, 16 cases (33.3%) were categorized as *HER2*-amplified. Of these, three, nine, and four were IHC 0/1+, IHC 2+, and IHC 3+, respectively. We observed ≥4 HER2 copies but <6 HER2 copies per nucleus in another six cases (12.5% of 48 polysomy 17 cases) categorized as HER2-equivocal, where one and five cases were IHC 0/1+ and IHC 2+, respectively. Of the 48 HER2-non-amplified cases, 26 were persistently HER2-non-amplified despite the CEP17 status (Table 2). Therefore, these findings demonstrate that there was discrepant interpretation of

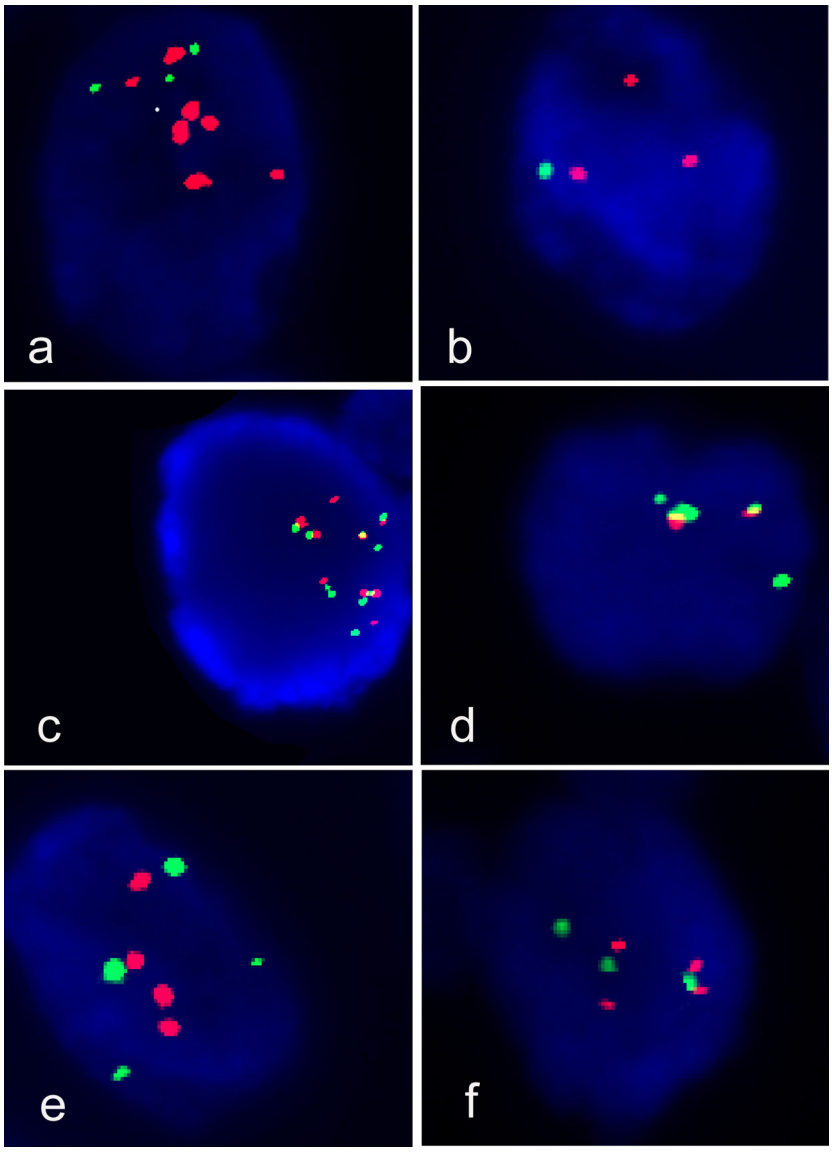

Fig. 1. FISH evaluation of HER2 status based on 2013 ASCO/CAP scoring criteria. (A and B) The 2013 ASCO/CAP criteria evaluate 20 tumor cells; a HER2/CEP17 ratio ≥ 2.0 is defined as *HER2* gene amplification. (C) HER2/CEP17 ratio < 2.0 but average HER2 copy number ≥ 6.0 is defined as HER2-positive. (D) HER2/CEP17 ratio < 2.0 and average HER2 copy number < 4.0 signals/cell is defined as HER2-negative. (E and F) Previous HER2-negative cases defined based on the 2007 ASCO/CAP guideline were redefined as HER2-amplified. A HER2/CEP17 ratio < 2.0 and average HER2 copy number ≥ 4.0 and <6.0 signals/cell was defined as HER2-equivocal. Red, HER2; green, CEP17. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

gene amplification status in 22 (12.6%) cases when the number of CEP17 copies was taken into account, and illustrates how breast cancer with polysomy 17 can be interpreted as HER2-positive, -equivocal, or -negative partly depending on which scoring method is applied to interpret the HER2 FISH results.

Discussion

Using FISH, we investigated the frequency of polysomy 17 and its association with HER2 alteration in patients with invasive breast cancer.

Table 1
HER2 FISH results based on 2007 and 2013 ASCO/CAP scoring criteria.

HER2 IHC results	Cases	2007 scoring criteria		2013 scoring criteria		*p*
0/1+	17	Negative	16	Negative	11	<0.05
		Equivocal	1	Equivocal	2	
		Positive	0	Positive	3	
2+	95	Negative	61	Negative	47	<0.05
		Equivocal	0	Equivocal	5	
		Positive	34	Positive	43	
3+	63	Negative	7	Negative	3	>0.05
		Equivocal	0	Equivocal	0	
		Positive	56	Positive	60	

Table 2
Analysis of HER2 FISH results of cases with polysomy 17 based on 2013 ASCO/CAP scoring criteria.

HER2 IHC results	Chromosome 17 polysomy cases	2013 scoring criteria
0/1+	6	2 1 3
2+	37	23 5 9
3+	5	1 0 4

As polysomy 17 is relatively common in breast carcinoma, it is possible that HER2 FISH results can be misinterpreted. In a recently published series, Vanden Bempt et al. reported that >40% of breast carcinomas harbor increased CEP17 copy numbers [32]. In our study, there was polysomy 17 in 57.1% (100/175) of primary invasive breast carcinoma cases. In 52 patients, polysomy 17 accompanied HER2 cluster amplification; the remaining 48 cases were defined as HER2-non-amplified based on the 2007 ASCO/CAP guideline. Therefore, this led to many the patients with polysomy 17 but non-HER2 cluster amplification losing the opportunity to receive targeted treatment. When we reevaluated the 48 cases that were HER2-non-amplified and polysomy 17-accompanied, we found that 16 and six cases could be defined as HER2-amplified and HER2-equivocal, respectively. Compared to other cases, polysomy 17 was much more common in IHC 2+ cases, which agrees the findings of others [27,28,30]. Subsequently, there was a significant increase in the number of HER2-amplified and HER2-equivocal cases. Importantly, the majority of IHC 2+ cases, i.e., cases where there was an increase from 34 to 43 patients, were responsive to the targeted therapy, followed by the IHC 3+ cases; the reevaluation also improved the prospects for the IHC 0/1+ cases. In addition to the 16 cases redefined as HER2-amplified, redefining the six cases as HER2-equivocal means that these patients may be able to receive targeted treatment.

In our series, polysomy 17 was defined as CEP17/nucleus ratio > 1.86 [27–31], and we believe that CEP17 represents chromosome 17, but the question of whether CEP17 copy number actually reflects the condition of polysomy 17 remained. In view of this, determining HER2 amplification status may partly depend on whether CEP17 copy number is taken into account. Indeed, 54.2% of the cases harboring CEP17 did not have *HER2* gene amplification. Importantly, the majority of these cases had a borderline IHC score (2+), and >75% of patients who were IHC 2+ were HER2-negative by FISH. Therefore, these cases were not responsive to anti-HER2 targeted therapy and did not fit the category of HER2-amplified breast carcinoma.

Another interesting issue of clinical relevance is whether polysomy 17 is associated with clinical behavior similar to that of HER2-amplified tumors. Many previous studies suggest that independently of HER2 amplification status, the presence of CEP17 alterations identifies a subset of breast cancer with more aggressive biological and clinical behaviors that may not respond to conventional therapy [30,33–35]. In a recent study, Bartlett et al. showed that the presence of polysomy 17, as established by CEP17 FISH, was predictive of response to anthracyclines [36]. Therefore, it is important to assess chromosome 17 copy number to investigate its possible implication in the clinical management of patients with invasive primary breast cancer. Indeed, a recently published study suggested that the presence of CEP17 alterations could identify a more aggressive subset of breast cancers that are non-responsive to conventional therapy independently of HER2 amplification status [37]. However, other researchers believe that polysomy 17 without HER2 amplification do not predict response to lapatinib in metastatic breast cancer [38]. Therefore, long-term studies are required to determine the relationship between the role of polysomy 17 in prognosis or the clinical response to trastuzumab in breast cancer.

In summary, depending on which criterion is used for interpretation, polysomy 17 is a crucial cause of misinterpretation of HER2 FISH results. Using the 2013 ASCO/CAP scoring criteria evaluate HER2 status resulted in a significantly higher number of HER2-amplified cases being identified, especially IHC 2+ cases, which identifies more patients appropriate for targeted treatment. However, as there are no methods to determine chromosome 17 status precisely, determining what CEP17 amplification means in terms of response to trastuzumab and anthracycline treatment requires further study.

References

[1] J. Ferlay, H.R. Shin, F. Bray, D. Forman, C. Mathers, D.M. Parkin, Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, Int. J. Cancer 127 (12) (2010) 2893–2917.
[2] C.E. Geyer, J. Forster, D. Lindquist, S. Chan, C.G. Romieu, T. Pienkowski, A. Jagiello-Gruszfeld, J. Crown, A. Chan, B. Kaufman, et al., Lapatinib plus capecitabine for HER2-positive advanced breast cancer, N. Engl. J. Med. 355 (26) (2006) 2733–2743.
[3] E.A. Perez, Cardiac toxicity of ErbB2-targeted therapies: what do we know? Clin. Breast Cancer 8 (suppl. 3) (2008) S114–S120.
[4] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, et al., Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (11) (2001) 783–792.
[5] B. Cetin, M. Benekli, F. Dane, C. Boruban, M. Gumus, B. Oksuzoglu, M.A. Kaplan, G. Tufan, A. Sevinc, U. Coskun, et al., Lapatinib plus capecitabine for HER2-positive advanced-stage breast cancer in elderly women: review of the Anatolian Society of Medical Oncology (ASMO) experience, Breast Care 8 (1) (2013) 67–70.
[6] A. Ardavanis, P. Kountourakis, F. Kyriakou, S. Malliou, I. Mantzaris, A. Garoufali, I. Yiotis, A. Scorilas, N. Baziotis, G. Rigatos, Trastuzumab plus paclitaxel or docetaxel in HER-2-negative/HER-2 ECD-positive anthracycline- and taxane-refractory advanced breast cancer, Oncologist 13 (4) (2008) 361–369.
[7] S. Verma, D. Miles, L. Gianni, I.E. Krop, M. Welslau, J. Baselga, M. Pegram, D.Y. Oh, V. Dieras, E. Guardino, et al., Trastuzumab emtansine for HER2-positive advanced breast cancer, N. Engl. J. Med. 367 (19) (2012) 1783–1791.
[8] M.F. Press, R.S. Finn, D. Cameron, A. Di Leo, C.E. Geyer, I.E. Villalobos, A. Santiago, R. Guzman, A. Gasparyan, Y. Ma, et al., HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer, Clin. Cancer Res. 14 (23) (2008) 7861–7870.
[9] L. Gianni, A. Llado, G. Bianchi, J. Cortes, P.L. Kellokumpu-Lehtinen, D.A. Cameron, D. Miles, S. Salvagni, A. Wardley, J.C. Goeminne, et al., Open-label, phase II, multicenter, randomized study of the efficacy and safety of two dose levels of Pertuzumab, a human epidermal growth factor receptor 2 dimerization inhibitor, in patients with human epidermal growth factor receptor 2-negative metastatic breast cancer, J. Clin. Oncol. 28 (7) (2010) 1131–1137.
[10] M. Hubalek, C. Brunner, K. Mattha, C. Marth, Resistance to HER2-targeted therapy: mechanisms of trastuzumab resistance and possible strategies to overcome unresponsiveness to treatment, Wiener Medizinische Wochenschrift 160 (19–20) (2010) 506–512.
[11] M. Dowsett, J. Houghton, C. Iden, J. Salter, J. Farndon, R. A'Hern, R. Sainsbury, M. Baum, Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status, Ann. Oncol. 17 (5) (2006) 818–826.
[12] A.S. Knoop, S.M. Bentzen, M.M. Nielsen, B.B. Rasmussen, C. Rose, Value of epidermal growth factor receptor, HER2, p53, and steroid receptors in predicting the efficacy of tamoxifen in high-risk postmenopausal breast cancer patients, J. Clin. Oncol. 19 (14) (2001) 3376–3384.
[13] Y.W. Moon, S. Park, J.H. Sohn, D.R. Kang, J.S. Koo, H.S. Park, H.C. Chung, B.W. Park, Clinical significance of progesterone receptor and HER2 status in estrogen receptor-positive, operable breast cancer with adjuvant tamoxifen, J. Cancer Res. Clin. Oncol. 137 (7) (2011) 1123–1130.
[14] C.A. Parise, K.R. Bauer, M.M. Brown, V. Caggiano, Breast cancer subtypes as defined by the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) among women with invasive breast cancer in California, 1999–2004, Breast J. 15 (6) (2009) 593–602.
[15] S. Chia, B. Norris, C. Speers, M. Cheang, B. Gilks, A.M. Gown, D. Huntsman, I.A. Olivotto, T.O. Nielsen, K. Gelmon, Human epidermal growth factor receptor 2 overexpression as a prognostic factor in a large tissue microarray series of node-negative breast cancers, J. Clin. Oncol. 26 (35) (2008) 5697–5704.

[16] J.S. Ross, E.A. Slodkowska, W.F. Symmans, L. Pusztai, P.M. Ravdin, G.N. Hortobagyi, The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine, Oncologist 14 (4) (2009) 320–368.
[17] M.J. Piccart-Gebhart, M. Procter, B. Leyland-Jones, A. Goldhirsch, M. Untch, I. Smith, L. Gianni, J. Baselga, R. Bell, C. Jackisch, et al., Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer, N. Engl. J. Med. 353 (16) (2005) 1659–1672.
[18] E.H. Romond, E.A. Perez, J. Bryant, V.J. Suman, C.E. Geyer Jr., N.E. Davidson, E. Tan-Chiu, S. Martino, S. Paik, P.A. Kaufman, et al., Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer, N. Engl. J. Med. 353 (16) (2005) 1673–1684.
[19] E.J. Bowles, R. Wellman, H.S. Feigelson, A.A. Onitilo, A.N. Freedman, T. Delate, L.A. Allen, L. Nekhlyudov, K.A. Goddard, R.L. Davis, et al., Risk of heart failure in breast cancer patients after anthracycline and trastuzumab treatment: a retrospective cohort study, J. Natl. Cancer Inst. 104 (17) (2012) 1293–1305.
[20] S. Paik, C. Kim, N. Wolmark, HER2 status and benefit from adjuvant trastuzumab in breast cancer, N. Engl. J. Med. 358 (13) (2008) 1409–1411.
[21] S.D. Russell, K.L. Blackwell, J. Lawrence, J.E. Pippen Jr., M.T. Roe, F. Wood, V. Paton, E. Holmgren, K.W. Mahaffey, Independent adjudication of symptomatic heart failure with the use of doxorubicin and cyclophosphamide followed by trastuzumab adjuvant therapy: a combined review of cardiac data from the National Surgical Adjuvant breast and Bowel Project B-31 and the North Central Cancer Treatment Group N9831 clinical trials, J. Clin. Oncol. 28 (21) (2010) 3416–3421.
[22] M. Bilous, C. Ades, J. Armes, J. Bishop, R. Brown, B. Cooke, M. Cummings, G. Farshid, A. Field, A. Morey, et al., Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER2000 International Study, Breast 12 (2) (2003) 92–98.
[23] R.J. Zarbo, M.E. Hammond, Conference summary, Strategic Science symposium. Her-2/neu testing of breast cancer patients in clinical practice, Arch. Pathol. Lab. Med. 127 (5) (2003) 549–553.
[24] A.C. Wolff, M.E. Hammond, D.G. Hicks, M. Dowsett, L.M. McShane, K.H. Allison, D.C. Allred, J.M. Bartlett, M. Bilous, P. Fitzgibbons, et al., Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update, J. Clin. Oncol. 31 (31) (2013) 3997–4013.
[25] L.P. Middleton, K.M. Price, P. Puig, L.J. Heydon, E. Tarco, N. Sneige, K. Barr, M.T. Deavers, Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases, Arch. Pathol. Lab. Med. 133 (5) (2009) 775–780.
[26] A.C. Wolff, M.E. Hammond, D.F. Hayes, Re: predictability of adjuvant trastuzumab benefit in N9831 patients using the ASCO/CAP HER2-positivity criteria, J. Natl. Cancer Inst. 104 (12) (2012) 957–958.
[27] M. Cuadros, P. Talavera, F.J. Lopez, I. Garcia-Perez, A. Blanco, A. Concha, Real-time RT-PCR analysis for evaluating the Her2/neu status in breast cancer, Pathobiology 77 (1) (2010) 38–45.
[28] C.L. Hyun, H.E. Lee, K.S. Kim, S.W. Kim, J.H. Kim, G. Choe, S.Y. Park, The effect of chromosome 17 polysomy on HER-2/neu status in breast cancer, J. Clin. Pathol. 61 (3) (2008) 317–321.
[29] H. Jiang, X. Bai, F. Meng, C. Zhang, X. Zhang, Evaluation of chromosome 17 polysomy in breast cancer by FISH analysis of whole nuclei, and its clinicopathological significance, Oncol. Lett. 7 (6) (2014) 1954–1958.
[30] U. Krishnamurti, J.L. Hammers, F.D. Atem, P.D. Storto, J.F. Silverman, Poor prognostic significance of unamplified chromosome 17 polysomy in invasive breast carcinoma, Mod. Pathol. 22 (8) (2009) 1044–1048.
[31] Y. Liu, L. Ma, D. Liu, Z. Yang, C. Yang, Z. Hu, W. Chen, Z. Yang, S. Chen, Z. Zhang, Impact of polysomy 17 on HER2 testing of invasive breast cancer patients, Int. J. Clin. Exp. Pathol. 7 (1) (2014) 163–173.
[32] I. Vanden Bempt, P. Van Loo, M. Drijkoningen, P. Neven, A. Smeets, M.R. Christiaens, R. Paridaens, C. De Wolf-Peeters, Polysomy 17 in breast cancer: clinicopathologic significance and impact on HER-2 testing, J. Clin. Oncol. 26 (30) (2008) 4869–4874.
[33] S.J. Lim, A. Cantillep, P.M. Carpenter, Validation and workflow optimization of human epidermal growth factor receptor 2 testing using INFORM HER2 dual-color in situ hybridization, Hum. Pathol. 44 (11) (2013) 2590–2596.
[34] C.B. Moelans, J.S. Reis-Filho, P.J. van Diest, Implications of rarity of chromosome 17 polysomy in breast cancer, Lancet Oncol. 12 (12) (2011) 1087–1089.
[35] W. Zhang, Y. Yu, The important molecular markers on chromosome 17 and their clinical impact in breast cancer, Int. J. Mol. Sci. 12 (9) (2011) 5672–5683.
[36] A.I. Bartlett, J. Starcyznski, T. Robson, A. Maclellan, F.M. Campbell, C.J. van de Velde, A. Hasenburg, C. Markopoulos, C. Seynaeve, D. Rea, et al., Heterogeneous HER2 gene amplification: impact on patient outcome and a clinically relevant definition, Am. J. Clin. Pathol. 136 (2) (2011) 266–274.
[37] A.D. Watters, J.J. Going, T.G. Cooke, J.M. Bartlett, Chromosome 17 aneusomy is associated with poor prognostic factors in invasive breast carcinoma, Breast Cancer Res. Treat. 77 (2) (2003) 109–114.
[38] C.B. Moelans, R.A. de Weger, P.J. van Diest, Chromosome 17 polysomy without HER2 amplification does not predict response to lapatinib in metastatic breast cancer – letter, Clin. Cancer Res. 16 (24) (2010) 6177, author reply 6178.